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Evolution of mollusc lens crystallins: Glutathione S-transferase/S-crystallins and aldehyde dehydrogenase/Ω-crystallins*

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Abstract: Diverse crystallins (abundant water-soluble proteins) are responsible for the optical properties of transparent cellular eye lenses and are multifunctional proteins that have been recruited from stress proteins and enzymes by enhanced lens expression. The major (S-crystallins) and minor (Ω-crystallin) cephalopod crystallins were recruited from glutathione S-transferase (GST) and aldehyde dehydrogenase (ALDH), respectively. S-crystallins underwent multiple gene duplications while Ω-crystallin appears to be encoded in a single-copy gene. Except for one S-crystallin (considered a "molecular fossil"), S-crystallins lack enzyme activity due to mutation and insertion of a variable central peptide by exon shuffling. The Ω-crystallin is the sole crystallin in scallops. Scallop Ω-crystallin does not bind the co-factor NAD^+/NADH, lacks enzyme activity, and is a tetramer but migrates as a dimer by gel filtration, suggesting structural adaptations for crystallin function. Similar transcription factors (Pax6 among others) appear to drive high lens expression of crystallin genes in molluscs and other species consistent with convergent recruitment of the non-homologous crystallin genes.

Key words: cephalopods, scallops, enzymes, lens proteins, eye, gene expression

Cephalopods and scallops have camera-type, lens-containing eyes that appear grossly like the complex eyes of vertebrates. Indeed, the similarities in the overall structures and the common use of opsin family members for phototransduction within the photoreceptors have made the camera-type eyes of molluscs and vertebrates prototypical examples of convergent (independent) evolution of specialized organs (Packard 1972). However, studies showing that invertebrates and vertebrates employ similar transcription factors (especially Pax6) for eye development re-opened the idea that diverse eyes are monophyletic (derived once and are thus homologous due to common ancestry) rather than polyphyletic (derived independently multiple times) (Gehring and Ikeo 1999, Gehring 2004). Although the similarities in their transcriptional networks for eye development favored a single origin for eyes throughout the animal kingdom, it is not proof of monophyletic eye evolution for various reasons, including the facts that the eye developmental networks show differences among species, the networks are not used exclusively for one tissue or organ, and there are major developmental differences in eye development in different species, especially between invertebrates and vertebrates. In brief then, the existing data are generally consistent with a great deal of parallel evolution (independent recruitment of similar networks of genes) among the diverse eyes of different species.

Analysis of lens crystallins provides a detailed view of independent processes during eye evolution. Crystallins comprise 80-90% of the water-soluble proteins of transparent cellular lenses and are responsible for their optical properties (Bloemendal and de Jong 1991). The crystallins are diverse proteins that, despite their common function for lens refraction, often differ among taxonomic groups, *i.e.*, many crystallins are taxon-specific (Wistow and Piatigorsky 1988). In addition to being highly expressed in the lens, crystallins are often present in lower concentrations in other tissues where they serve non-refractive roles. Surprisingly, the taxon-specific crystallins are generally (not exclusively) identical or related to metabolic enzymes and consequently are called enzyme-crystallins (Wistow and Piatigorsky 1988, de Jong *et al.* 1989). The use of the identical protein for a refractive role in the lens and for one or more non-refractive roles in other tissues (as well as in the lens) has been called gene sharing (Piatigorsky *et al.* 1988) to convey the situation of having two or more distinct molecular functions directed by (*i.e.*, sharing) the identical protein-coding gene sequence (Piatigorsky and Wistow 1989, Piatigorsky 2007).

Many lens crystallins of invertebrates and vertebrates display gene sharing since they have non-refractive functions outside of the lens in addition to their refractive functions in the lens (Tomarev and Piatigorsky 1996). The present communication reviews the lens crystallins of the camera-type

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eyes of cephalopods and scallops. The existing data are consistent with these molluscan crystallins being recruited from the metabolic enzymes glutathione S-transferase (GST) and aldehyde dehydrogenase (ALDH). Many diverse proteins have been recruited to be crystallins in different species in vertebrates and invertebrates (Figs. 1 and 2).

S-Crystallins

S-crystallins were named as such because they are the most abundant proteins in the squid lens (Siezen and Shaw 1982). S-crystallins are a large family of enzyme-crystallins that are homologous to the metabolic enzyme GST and are highly specialized for lens function (Tomarev and Zinovieva 1988, Tomarev *et al.* 1991, Tomarev *et al.* 1993, Chiou *et al.* 1995). They are differentially expressed in a radial gradient consistent with their expected role in focusing by creation of a refractive index gradient (Sweeney *et al.* 2007). The squid (Tomarev *et al.* 1992) and octopus (Tomarev *et al.* 1991) S-crystallin mRNAs are expressed strictly in the lens except that a few are also highly expressed in the squid cornea (Cuthbertson *et al.* 1992), consistent with the concept of corneal crystallins (Piatigorsky 1998, Jester *et al.* 1999, Piatigorsky 2001). The optical role of corneal crystallins, so named because of their abundance, remains elusive (Nees *et al.* 2002, Jester *et al.* 2005, Estey *et al.* 2007).

The cephalopod digestive gland expresses authentic GST that has high enzymatic activity; this active enzyme is expressed barely if at all in the lens (Harris *et al.* 1991, Tomarev *et al.* 1993, Tang *et al.* 1994). The three-dimensional structure of squid GST (known as GST σ) indicates that it has an unusually open active site that correlates with its high activity and has a characteristic dimer interface that differs from that of the vertebrate GST isoforms (Ji *et al.* 1995). By contrast with the digestive gland active GST σ, all but one (SL11 in squid; Lops4 in octopus) of the S-crystallins that have been examined lack enzyme activity (Tomarev *et al.* 1995).

The GST-related S-crystallin gene family (>20 members) was derived by gene duplications (Tomarev and Zinovieva 1988, Tomarev *et al.* 1992). The S-crystallins lost

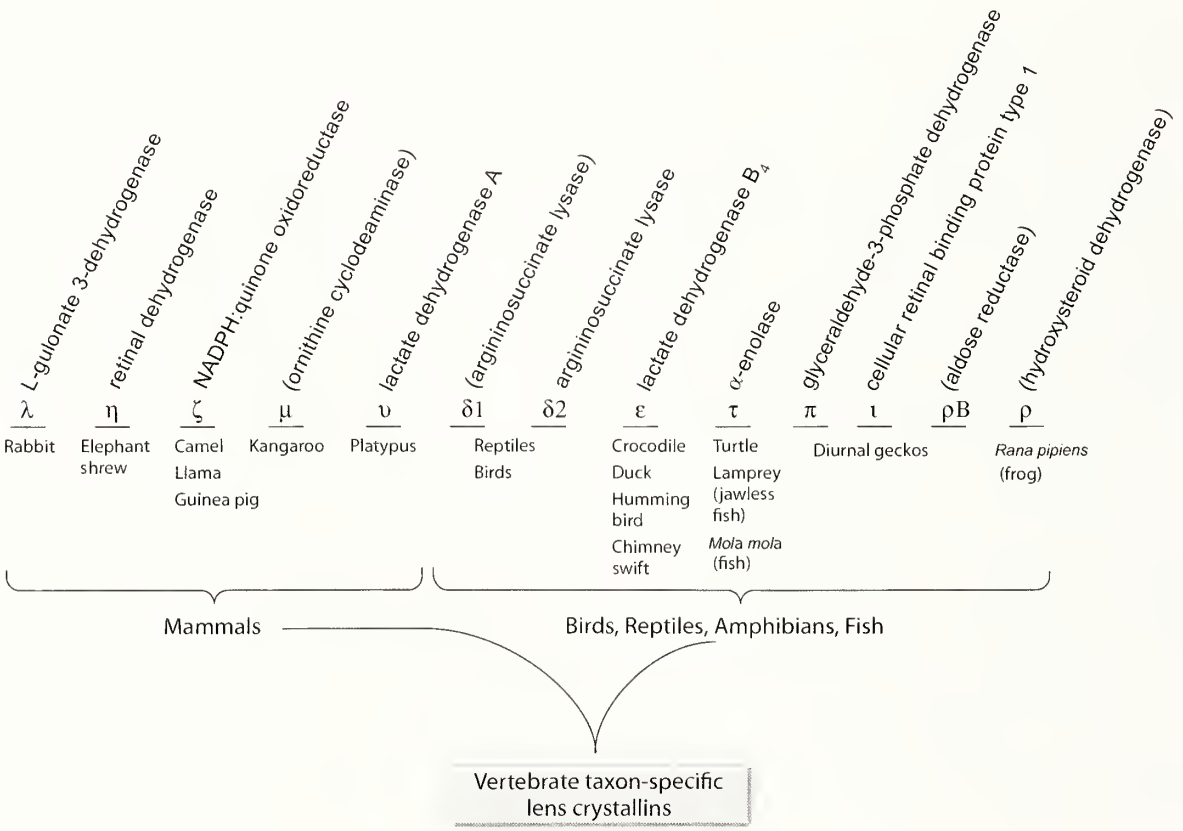

Figure 1. Vertebrate taxon-specific lens crystallins. Enzymes in parenthesis lack activity. (Reprinted by permission of the publisher from *Gene Sharing and Evolution: The Diversity of Protein Functions* by Joram Piatigorsky, p. 62, Harvard University Press, Cambridge, Massachusetts, © 2007 by the President and Fellows of Harvard College.)

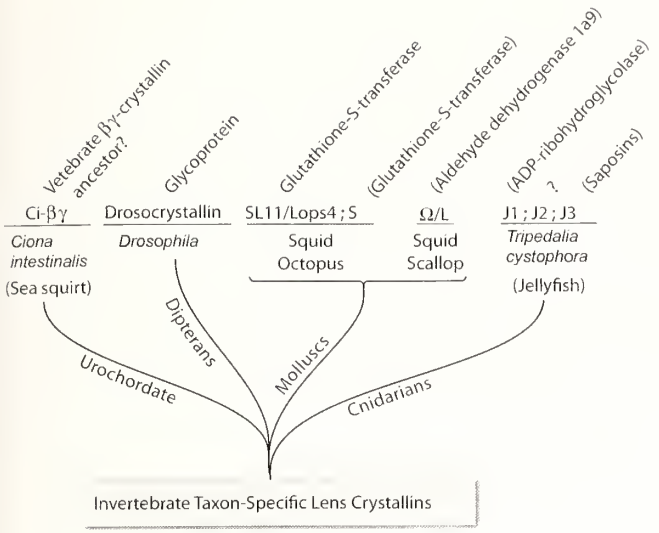

Figure 2. Invertebrate taxon-specific lens crystallins. Ci-βγ-crystallin in urochordates may be an ancestor of the vertebrate-βγ-crystallins (Shimeld *et al.* 2005, Piatigorsky 2006); however, *Ciona intestinalis* does not have a lens in its larval eye (there is no eye in the adult). Thus, the function of Ci-βγ-crystallin is not known. Enzymes in parenthesis lack activity. (Reprinted by permission of the publisher from *Gene Sharing and Evolution: The Diversity of Protein Functions* by Joram Piatigorsky, p. 64, Harvard University Press, Cambridge, Massachusetts. © 2007 by the President and Fellows of Harvard College.)

enzyme activity due to the insertion of an additional exon encoding peptides of variable length within the genes and to multiple point mutations sustained throughout the coding region of the genes. Only the squid SL11 gene and its orthologous gene in the octopus, Lops4, do not contain an additional central peptide acquired by exon insertion, as in the other S-crystallin genes; their encoded proteins are thus more closely related to the enzymatically active GSTσ than are the other S-crystallin proteins. Consequently SL11/Lops4 has residual GST activity (although much lower than GSTσ) despite being lens-specific. It is interesting, but speculative, to think of the SL11/Lops4 gene as a "molecular living fossil" of the enzyme-crystallin recruitment process during evolution, representing the original GST duplicate whose expression was directed to the cephalopod lens (Tomarev *et al.* 1995, Tomarev and Piatigorsky 1996).

Ω-Crystallin

Ω-crystallins of molluscs have been recruited from one or more members of the large group of aldehyde dehydrogenases (ALDHs) which metabolize a wide number of endogenous and exogenous aldehydes (Perozich *et al.* 1999, Vasiliou *et al.* 1999, Sophos and Vasiliou 2003). ALDHs are very ancient and found ubiquitously in microbes, plants, and animals. In eukaryotes alone, the ALDHs comprise at least 20 gene families. As a general rule, ALDHs with less than 40% identity in amino acid sequence are placed in different families and those with more than 60% identity belong to the same subfamily.

Due to the extensive number of ALDH genes, many showing high sequence identity and extensive gene conversions, their exact relationships to one another and time of gene duplications during evolution are uncertain. Ω-crystallin is a minor crystallin in octopus (ALDH1C1) and squid (ALDH1C2) lenses (Chiou 1988, Zinovieva *et al.* 1993) and is the only crystallin in the scallop lens (ALDH1A9), where it comprises ~70% of the water-soluble protein (Piatigorsky *et al.* 2000). It is also the sole crystallin protein present in the lens of the light organ of the squid *Euprymna scolopes* where it is known as L-crystallin (Montgomery and McFall-Ngai 1992). It is noteworthy that aldehyde dehydrogenase is the only enzyme known to have been recruited as a lens crystallin in both vertebrates and invertebrates and as a corneal crystallin in vertebrates (Tomarev and Piatigorsky 1996, Piatigorsky 1998). In vertebrates, the lenses of elephant shrews contain η-crystallin (ALDH1A8) (Graham *et al.* 1996), and ALDH3A1 and ALDH1A1 are the predominant corneal crystallins of mammals (Abedinia *et al.* 1990, Piatigorsky 1998, Jester *et al.* 2005). Although η-crystallin has retinaldehyde dehydrogenase activity (Graham *et al.* 1996), mollusc Ω-crystallins lack enzyme activity (Montgomery and McFall-Ngai 1992, Zinovieva *et al.* 1993, Piatigorsky *et al.* 2000). A mutation changing a critical cysteine to arginine in the active site of cephalopod Ω-crystallins has occurred, consistent with enzymatic inactivity of the encoded crystallin. Unexpectedly however, the active site sequence, including the critical cysteine, of the inactive scallop Ω-crystallin is intact. The reason for the absence of enzymatic activity of scallop Ω-crystallin appears to be due to the fact that it does not bind the required co-factor NAD^+ or NADH (Piatigorsky *et al.* 2000).

In contrast to the S-crystallin mRNAs in cephalopods, scallop Ω-crystallin mRNA is expressed weakly outside of the lens (gills, muscle) (Piatigorsky *et al.* 2000). Non-lens expression of Ω-crystallin is surprising because this abundant lens protein appears specialized for its non-enzymatic crystallin function. It remains possible that there are two closely related Ω-crystallin genes in the scallop, with the Ω-crystallin gene expressed solely in the lens and its duplicate expressed in other tissues. It is also possible that scallop Ω-crystallin will bind NAD^+ and has enzyme activity under different physiological conditions or can utilize an unknown substrate, allowing it to act as an enzyme under some circumstances. ALDHs are known to have wide substrate specificities as well as having non-enzymatic functions (Sophos

and Vasiliou 2003, Estey *et al.* 2007). Thus, further investigations are necessary to reveal the possible enzymatic and/or other non-refractive function(s) of Ω-crystallin.

The squid and octopus Ω-crystallins have 78% amino acid sequence identity to scallop Ω-crystallin and 56-58% amino acid sequence identities to cytoplasmic ALDH1A1 and mitochondrial ALDH2 of vertebrates, while the scallop Ω-crystallin is 64% and 67% identical to human cytoplasmic ALDH1A1 and mitochondrial ALDH2, respectively (see Horwitz *et al.* 2006). Despite the close similarities of the cephalopod and scallop Ω-crystallins to both cytoplasmic ALDH1 and mitochondrial ALDH2 enzymes, phylogenetic tree analysis shows that the cephalopod Ω-crystallins cluster with the cytoplasmic ALDH1 proteins and the scallop Ω-crystallin clusters with the mitochondrial ALDH2 proteins (Horwitz *et al.* 2006).

Despite the sequence homology of scallop Ω-crystallin to ALDH1 and ALDH2, the native protein migrates as a homodimer (100 kD) by gel filtration chromatography rather than a homotetramer (200 kD) as expected for an ALDH1 or ALDH2 (Piatigorsky *et al.* 2000). Re-examination of scallop Ω-crystallin by on-line multi-angle laser light scattering showed, however, that Ω-crystallin is a tetrameric protein despite its chromatographic properties (Horwitz *et al.* 2006). It, thus, appears as if the anomalous gel filtration chromatography behavior of scallop Ω-crystallin reflects structural alterations, yet to be discovered, associated with its specialized crystallin function. In that connection, X-ray crystallographic studies of elephant shrew η-crystallin provided direct evidence for numerous structural features indicating that this recruited ALDH-crystallin displays characteristics of the cytoplasmic ALDH1 as well as of mitochondrial ALDH2 (Bateman *et al.* 2003). Mollusc Ω-crystallins await crystallographic studies.

Finally, mollusc Ω-crystallins lack an N-terminal leader sequence directing the protein to mitochondria (Piatigorsky *et al.* 2000, Horwitz *et al.* 2006). The absence of an N-terminal leader sequence is unexpected for scallop Ω-crystallin because, in contrast to squid and octopus Ω-crystallins, the phylogenetic tree analysis suggests that it was recruited from a mitochondrial ALDH2-like enzyme. All known mitochondrial ALDH proteins have a mitochondrial leader sequence, unlike the cytoplasmic ALDH1 proteins that do not (Piatigorsky *et al.* 2000, Horwitz *et al.* 2006). The absence of a mitochondrial leader peptide allows Ω-crystallins to fill the cytoplasm and serve refractive crystallin roles rather than be shunted into the mitochondria. Similarly, η-crystallin of elephant shrews lacks a mitochondrial signal sequence and is a cytoplasmic, ALDH1 protein (Graham *et al.* 1996). Cephalopod and scallop Ω-crystallins also have numerous sequence differences whose biological significances remain to be elucidated, as discussed elsewhere (Horwitz *et al.* 2006).

Crystallin gene expression

The hallmark of a water-soluble protein being a crystallin is abundance in the transparent lens. It has been suggested that 5% of the water-soluble protein of the lens be considered a minimum concentration for a crystallin (de Jong *et al.* 1994). Crystallin genes have complex patterns for expression in numerous tissues as well as being specialized for high lens expression consistent with their having been recruited from proteins with non-crystallin roles presumably by undergoing a period of gene sharing during evolution. Moreover, crystallin gene expression in the lens is exquisitely regulated to create a smooth gradient of protein concentration that is highest in the center of the lens and lowest at the periphery. Cephalopod crystallins amount to as much as 70% of the protein in the center of the hard lenses. Scallop crystallins have a much lower concentration as judged by the softness of the scallop lenses (personal experience). The gradient of protein concentration in the lens produces a corresponding gradient of refractive index allowing a focused image of incident light upon the retinal photoreceptors. In addition, the crystallin concentrations within the individual lens cells must be regulated to minimize refractive index fluctuations so that the cells are transparent (Benedek 1971, Bettelheim and Siew 1983, Delaye and Tardieu 1983).

Little is known about crystallin gene regulation in cephalopods or scallops; therefore, it is useful to apply our knowledge about the well studied mechanisms of crystallin gene regulation in vertebrates. Investigations on vertebrates have found that lens-specific expression of the diverse, non-homologous genes is regulated by a similar set of transcription factors that are involved in lens development (Cvekl and Piatigorsky 1996, Duncan *et al.* 2004). Foremost among them is Pax6, which plays a key role in lens development of all vertebrate lenses (Gehring and Ikeo 1999, Gehring 2005). Additional factors include members of the Maf family, Sox2 and retinoic acid receptors, among others (Duncan *et al.* 2004, Piatigorsky 2007). Regulation of lens preferred expression of non-related crystallin genes by similar transcription factors implies convergent evolution of crystallin gene regulation. In short, the data indicate that, in vertebrates, the recruitment of diverse proteins (often with stress protective or enzymatic functions) to become lens crystallins occurred by independent modifications of their gene regulatory motifs (*cis*-control elements) making them accessible to a restricted set of transcription factors that direct lens development.

Although scant, the available data indicate that crystallin recruitment in invertebrates has also occurred by independent convergent changes in gene regulation. Both S-

crystallin and Ω-crystallin promoters have independently acquired DNA motifs that bind transcription factors from the same family that are used for vertebrate crystallin gene expression. A diagrammatic comparison of transcription factor binding sites in vertebrate and invertebrate crystallin promoters is shown in Fig. 3. Note that similar transcription factors (color-coded) bind to or activate the promoters of the non-homologous crystallin genes. Of particular interest are the Pax6 (PaxB in jellyfish) and Maf (MARE) binding sites on the various promoters since these transcription factors are critical for lens development. Functional studies showed that minimal squid promoter fragments for the SL20-1 crystallin gene and the SL11 gene are active in transfected rabbit lens epithelial cells (Tomarev *et al.* 1992). Promoter activity of these fragments depends upon AP-1 sites. AP-1 sites are also important for lens activity of vertebrate crystallin promoters (Piatigorsky and Zelenka 1992). The AP-1 sites in these squid crystallin promoters overlap with sequences similar to antioxidant and xenobiotic responsive elements which are involved in rat GST gene and guinea pig ξ-crystallin gene expression (Tomarev *et al.* 1992, 1994). GST and ξ-crystallin (which has quinine reductase activity) have physiological stress protective functions consistent with their genes being regulated by stress response elements and with the stress protective nature of vertebrate crystallin genes (de Jong *et al.* 1989, Horwitz 1992). The putative control elements of the S-crystallin promoters resemble the PL-1 and PL-2 (polyomavirus-like enhancer element 1 and polyomavirus-like enhancer element 2, respectively) *cis*-control elements of the lens-specific chicken βB1-crystallin promoter (Roth *et al.* 1991).

Although Pax6 appears necessary for establishing the eye field in cephalopods, it is uncertain at the present time whether or not it controls S-crystallin gene expression in the

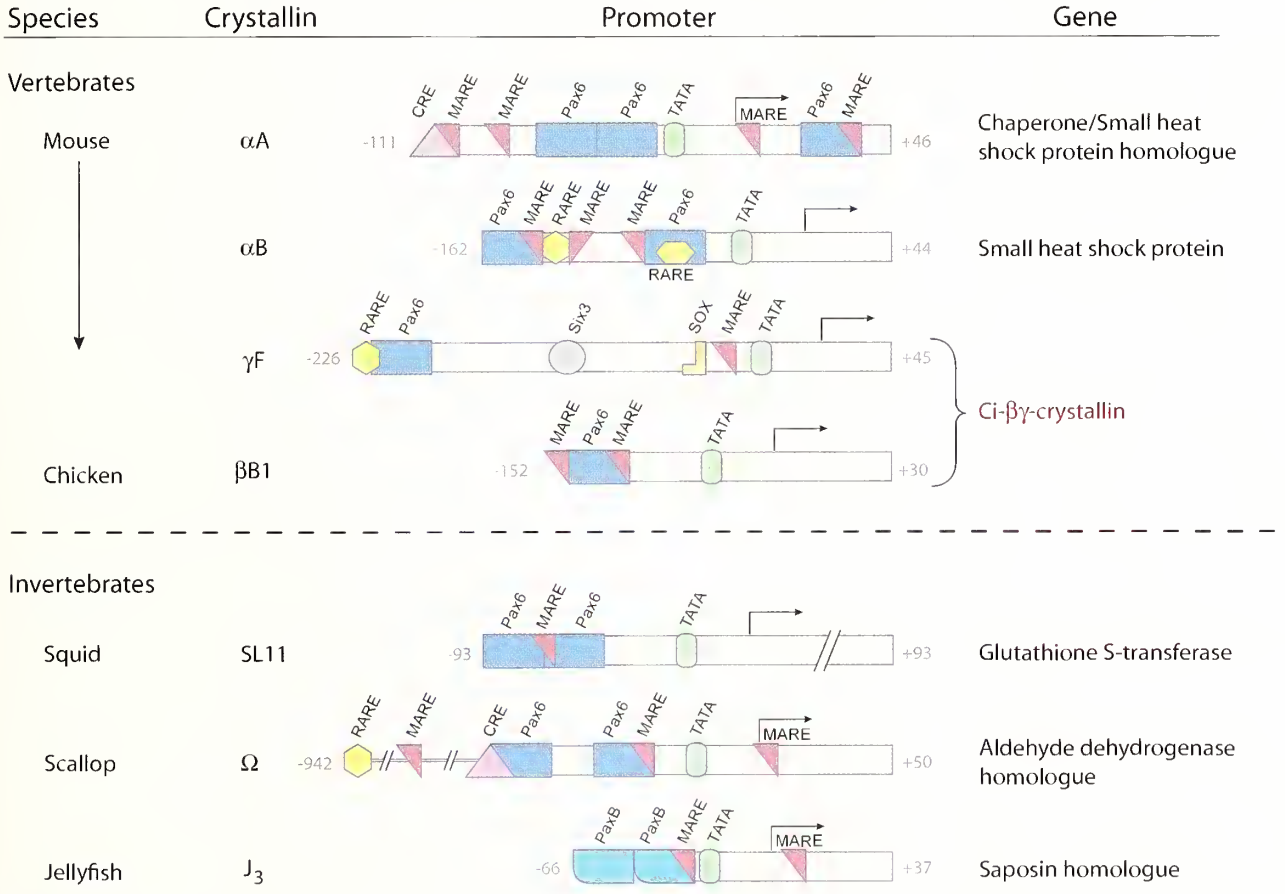

Figure 3. Diagrammatic representation of *cis*-control elements in the promoters of crystallin genes of vertebrates (upper) and invertebrates (lower). Note the similarity in control motifs in the non-homologous crystallin genes. CRE, cyclic AMP-responsive element; MARE, Maf regulatory element; RARE, retinoic acid receptor regulatory element. Jellyfish PaxB has a Pax2-like paired domain and a Pax6-like homeodomain. Ci-βγ-crystallin is a urochordate protein and may be ancestral to the vertebrate βγ-crystallins (Shimeld *et al.* 2005, Piatigorsky 2006). (Reprinted from Piatigorsky (2006) with permission from Nature Publishing Group.)

lens (Tomarev *et al.* 1997). A functional Pax6, as judged by its ability to induce ectopic eyes in *Drosophila*, is expressed in the anterior segment of the squid lens, consistent with it having a role in S-crystallin gene expression. However, neither the posterior lens cells nor the embryonic squid lentigenic cells express Pax6 (Tomarev *et al.* 1997), where S-crystallin synthesis is active (West *et al.* 1994). Nonetheless, potential Pax6 binding sites have been identified in the SL20-1 and SL-11 squid crystallin promoters [A. Cvekl (pers. comm.) in (Tomarev *et al.* 1995)].

Experiments with the scallop Ω-crystallin promoter extend the evidence that lens-preferred crystallin gene expression in molluscs is controlled by transcription factors that resemble those regulating lens-preferred crystallin gene expression in vertebrates (Carosa *et al.* 2002). The scallop Ω-crystallin promoter sequence contains an abundance of motifs that are similar to those in vertebrate crystallin promoters. These include *cis*-elements for oxidative stress response factors (CREB/Jun; AP-1) and developmental transcription factors (Pax6; Maf) as well as other gene regulatory proteins. Moreover, the results of co-transfection and site-specific mutagenesis experiments are consistent with overlapping CREB/Jun and Pax6 sites in the Ω-crystallin promoter being functional (Carosa *et al.* 2002). Thus, the "lens nature" of the scallop Ω-crystallin promoter is completely different from that of other ALDH promoters, which is striking in view of the fact that the scallop Ω-crystallin gene is homologous to and shares high sequence identity with ALDH1 and ALDH2 proteins (Carosa *et al.* 2002, Horwitz *et al.* 2006). Taken together, the data indicate that stress response and eye developmental transcription factors regulate lens-specific expression of mollusc S- and Ω-crystallin genes as they do vertebrate crystallin genes.

Does Pax2 regulate scallop Ω-crystallin gene expression?

Cubozoan jellyfish, members of the ancient Cnidarians, are particularly interesting with respect to the well-established role of Pax6 in the evolution and development of eyes throughout animals (Piatigorsky and Kozmik 2004). Cubozoan jellyfish have camera-type, lens-containing eyes as do cephalopods, scallops and vertebrates. The complex eyes of the cubozoan *Tripedalia cystophora* are situated on four equally spaced rhopalia nestled in open cavities on the surface bell surrounding the jellyfish (Piatigorsky *et al.* 1989, Piatigorsky and Kozmik 2004). Each sensory rhopalium contains two of these lens-containing eyes, one larger and one smaller, situated at right angles to one another as well as four simpler eyespots comprising photoreceptors that lack lenses. The rhopalia also have a relatively large statocyst used for orientation. The complex eyes have a mono-layered cornea and transparent, cellular lenses which refract incident light without spherical aberration, indicating the presence of a gradient of refractive index similar to vertebrate lenses (Nilsson *et al.* 2005). Three distinct crystallins (J1-, J2-, and J3-crystallin) (Piatigorsky *et al.* 1989), with J1 comprising three extremely similar polypeptides (Piatigorsky *et al.* 1993), are responsible for the optical properties of the lenses of the *T. cystophora*.

Despite having sophisticated eyes, jellyfish and other investigated Cnidaria, lack Pax6 (Sun *et al.* 1997, 2001, Groger *et al.* 2000, Miller *et al.* 2000). Instead, Cnidaria (as well as sponges (Hoshiyama *et al.* 1998)) have PaxB, which has a Pax2/5/8-like DNA binding paired domain and a Pax6-like homeodomain. It appears then as if modern Pax2 and Pax6 genes evolved from a PaxB-like ancestor by duplication and diversification in higher metazoans (Piatigorsky and Kozmik 2004). Indeed, PaxB of *Tripedalia cystophora* is a functional as well as structural hybrid of Pax2/5/8 and Pax6. For example, PaxB has the ability to induce small ectopic eyes in *Drosophila* as does both Pax2 and Pax6 (Kozmik *et al.* 2003). With respect to crystallin gene expression, jellyfish PaxB activates the jellyfish crystallin promoters in co-transfection tests although its paired domain will not recognize a Pax6 DNA binding site (Kozmik *et al.* 2003). Pax2, but not Pax6, is also capable of low-level activation of the jellyfish J3-crystallin promoter, consistent with the presence of Pax2-like *cis*-control elements that do not recognize a Pax6 paired domain. Crystallin genes of other species are activated by Pax6 but not by Pax2 since their promoters have Pax6 but lack Pax2 binding sites.

It is possible, although not known at present, that the scallop Ω-crystallin promoter is responsive to Pax2 as well as to Pax6. As mentioned above, this promoter has two putative Pax6 binding sites (Carosa *et al.* 2002). Inspection of the sequences of these Pax6 binding motifs reveals that site 2 of the scallop Ω-crystallin promoter has a 3' C consistent with preferential binding by Pax2. Moreover, a Pax2 cDNA has been cloned from scallop eyes (Kozmik and Piatigorsky, unpubl. data). It remains to be shown that Pax2 is expressed in the scallop lens and is capable of activating the Ω-crystallin promoter. A Pax2 contribution to the regulation on Ω-crystallin gene expression in scallop lenses would be very interesting and be consistent with convergent evolution of crystallin gene recruitment in scallops and jellyfish.

SUMMARY

Cephalopods and scallops have complex camera-type eyes. Their transparent lenses have recruited crystallins from the widely distributed families of metabolic GST and ALDH enzymes. Crystallin recruitment occurred by independent mutations of the *cis*-control elements of their genes allowing their promoters to be activated by transcription factors used

for lens development. This is the same convergent evolutionary process that has been used in recruitment of the vertebrate crystallin genes. The GST-derived/S-crystallin genes of cephalopods are expressed specifically in the lens and have undergone many gene duplications, insertions and base changes. Except for one S-crystallin, the cephalopod S-crystallins are enzymatically inactive proteins.

The cephalopod and scallop ALDH-derived/Ω-crystallins are encoded in single-copy genes. They show high sequence similarity to vertebrate cytoplasmic ALDH1 and mitochondrial ALDH2 proteins. Phylogenetic tree analysis indicates (but does not prove) that cephalopod Ω-crystallin was recruited from a cytoplasmic ALDH1-like gene while the scallop Ω-crystallin was recruited from a mitochondrial ALDH2-like gene. If scallop Ω-crystallin was indeed recruited from a mitochondrial ALDH2-like protein, it has lost its mitochondrial leader to resemble a cytoplasmic ALDH1-like protein. Neither cephalopod nor scallop Ω-crystallins show enzymatic activity, however for different reasons. The cephalopod Ω-crystallins have a mutated active site; by contrast scallop Ω-crystallin has a wild type active site sequence but the protein is unable to bind the NAD or NADH cofactor required for activity. The scallop Ω-crystallin gene is highly expressed in the lens but, unlike the cephalopod S-crystallin genes, is also expressed to a lesser extent in tissues outside of the eye. Scallop Ω-crystallin appears to have made adaptive changes in conformation affecting some of its properties. Similar to the S-crystallin promoters, the Ω-crystallin promoters have undergone independent sequence modifications making them responsive to transcription factors used for lens development in vertebrates, consistent with convergent evolution of crystallin gene recruitment.

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